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[54] **SCREENING NATURAL SAMPLES FOR NEW THERAPEUTIC COMPOUNDS USING CAPILLARY ELECTROPHORESIS**

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[52] **U.S. Cl.** **435/7.1**; 435/4; 435/5; 435/6; 435/7.8; 435/7.9; 435/7.2; 204/299; 204/180.1; 204/407; 356/344; 436/6

[58] **Field of Search** 435/7.1, 5, 4, 7.8, 435/7.9, 7.2, 6; 204/299, 180.1, 182.8, 407; 356/344; 436/536

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[57] **ABSTRACT**

A method in which natural sample components are simultaneously fractionated and screened for compounds that bind tightly to specific molecules of interest is disclosed. Such newly isolated ligands are good candidates for potential therapeutic or diagnostic compounds. The natural sample is first combined with a potential target molecule and then subjected to capillary electrophoresis (CE). Charged (or even neutral) compounds present in the natural sample that bind to the added target molecule can alter its normal migration time upon CE, by changing its charge-to-mass ratio, or will cause a variation in peak shape or area. Complex formation can be detected by simply monitoring the migration of the target molecule during electrophoresis. Any new ligands that bind to the target molecule will be good candidates for therapeutic or diagnostic compounds. Interfering, weak-binding ligands commonly present in crude extracts are not detected. Small, neutral ligands, as well as charged ligands, can be identified in competitive binding experiments with known, charged competitor molecules.

26 Claims, 12 Drawing Sheets

solution-based affinity assay to discover new active compounds, e.g., potential new drugs or diagnostic compounds, from complex biological material, particularly natural samples (NS). This method is able to overcome major problems associated with current pharmaceutical screens such as poor detection levels and low sensitivity caused by interfering compounds present in complex natural samples. Furthermore, the method of the invention is capable of uncovering drug activity in samples where such activity had previously been unobserved using other primary screening methods.

In the method of the invention, components of complex biological material, e.g., from natural samples, are simultaneously fractionated and screened for new compounds that bind tightly to specific molecules of interest. Such newly isolated ligands are good candidates for potential therapeutic or diagnostic compounds. This single-step assay requires very small amounts of sample and utilizes a highly specific, solution-based affinity assay, thus facilitating the identification of a true positive sample as well as avoiding interfering background components.

In one application of the invention, the sample of complex biological material, e.g., natural sample, is first combined with a known target molecule and then fractionated by CE, while the migration of the target molecule is tracked. Charged compounds present in the natural sample that bind tightly to the target molecule will alter its normal migration time or cause a change in peak shape or area by changing its charge-to-mass ratio or overall structure. Complex formation can be detected by simply tracking the target molecule during electrophoresis and comparing its migration pattern or electrophoretic profile in the presence of a natural sample to that in the absence of the sample. Any ligands that bind to the target molecule are candidates for therapeutic lead compounds or diagnostic compounds.

In another aspect of the method, small, neutral ligands as well as charged ligands can be identified in competitive binding experiments using known, charged competitor molecules. A competitor-target molecule complex or unbound competitor can be observed to have a specific migration time, as a reference standard. Any ligands in the natural sample that are bound tightly to the target molecule do not allow the competitor to interact with the target, thus changing the observed mobility of the target molecule.

Thus, in general, the method of the invention for screening complex biological material for new active compounds includes providing a sample of complex biological material, combining the sample with a target molecule, injecting a sample from the previous step into an apparatus for capillary electrophoresis, subjecting the sample to capillary electrophoresis and monitoring the migration of the labeled or unlabeled target molecule upon electrophoresis (either directly or indirectly). Preferably, the method also includes comparing the migration of the target to the migration of a reference standard. A reference standard is usually an analyte with a known migration time which is used as an internal control to determine whether the migration of the target or competitor ligand changed in the presence of complex biological sample. For example, the reference standard can be an excess of unbound target or competitor ligand-shifted target, an excess of competitor ligand, an independent non-interacting molecule, or even a range of time in which the target or competitor ligand normally runs in the absence of complex biological sample.

In a further application of the method of the invention, to subtractive analysis, all possible detectable compounds in a sample of complex biological material that is to be screened are detected by a specific method to serve as a reference standard, following capillary electrophoresis of the sample. An additional sample of the biological material to be screened is then combined with a potential target molecule, the combined material is fractionated by CE, and all compounds are again detected by the same detection method. Any compounds detected in the reference standard sample that are not among the compounds detected (by the same method) following electrophoresis of the combined material are candidate products for further analysis as new, useful ligands of the target molecule.

Once detected, the binding ligands may be isolated and tested for, e.g., their therapeutic efficacy and pharmacokinetic properties. With the use of dye-conjugated molecules and laser-induced fluorescence, the method of the invention provides the ability to detect ligand concentrations, directly in the sample, in the low nanomolar range, which is substantially lower than the micromolar concentrations that are the limit in most current natural product screens. In addition, washing of the capillary and replacement of the buffer or matrix in a capillary electrophoresis system is rapid and allows higher throughput of crude natural samples than is possible with standard procedures based on affinity chromatography.

The method of the invention will permit the rapid detection of potentially useful, new molecules in natural samples that escape standard screens due to low concentrations and/or the presence of interfering compounds. The small scale of CE has major advantages in that the quantity of rare or potentially hazardous assay components, e.g., the natural sample itself, the target molecule or the buffers used, can be reduced considerably.

The method of the invention will accommodate high-throughput screening of natural samples suitable for automation by employing multiple capillaries or multiple channels on microfabricated devices and several target molecules per channel or capillary. In many cases, on-line structural information of lead candidates can be directly ascertained by coupling a mass spectrometer, or other analytical device such as NMR, directly to the capillary or channel.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows one general model for isolating new ligands from natural samples according to the method of the invention;

FIG. 2*a* shows one model for detecting direct binding in the method of the invention;

FIG. 2*b* shows another model for detecting direct binding in the method of the invention;

FIG. 2*c* shows another model for detecting direct binding in the method of the invention;

FIG. 3 shows capillary electrophoresis of thrombin in the presence and absence of its natural inhibitor hirudin as an example of the model for the method of the invention shown in FIG. 2*a*;

FIG. 4 shows capillary electrophoresis of thrombin in the presence and absence of a thrombin-binding aptamer as an additional example of the model shown in FIG. 2*a*;

FIG. 5 shows capillary electrophoresis of thrombin in the presence of a thrombin-binding aptamer, with or without hirudin, as an example of the model shown in FIG. 2*c*;

other alterations to the compositions and methods set forth herein. It is therefore intended that the protection granted by Letters Patent hereon be limited only by the definitions contained in the appended claims and equivalents thereof.

What is claimed is:

1. A method of screening complex biological material for previously unidentified ligands of a selected target, said method comprising, in the order given, the steps of:

(1) providing a sample of complex biological material;

(2) combining said sample of complex biological material with said selected target to form a sample/target mixture;

(3) injecting an aliquot of said sample/target mixture from step (2) into an apparatus for capillary electrophoresis without sieving matrix;

(4) subjecting said aliquot of said sample/target mixture to capillary electrophoresis without sieving matrix;

(5) tracking the migration of said target upon said capillary electrophoresis;

(6) determining whether the migration pattern of said target from step (5) indicates the presence of a candidate unidentified ligand in said sample of complex biological material;

(7) isolating said candidate compound from said complex biological material; and

(8) determining whether said isolated compound has not been previously identified as interacting with said target.

2. The method of claim 1, wherein said step (6) comprises:

comparing the migration of said target to a reference standard comprising the migration time for an aliquot of said target alone to reach a detection point in said capillary electrophoresis apparatus.

3. The method of claim 1, wherein said step (6) comprises:

comparing the migration pattern of said target to a reference standard comprising the migration pattern of an aliquot of said target alone.

4. A method of screening complex biological material for previously unidentified ligands of a selected target, said method comprising, in the order given, the steps of:

(1) providing a first sample of complex biological material;

(2) injecting said first sample of complex biological material into an apparatus for capillary electrophoresis without sieving matrix;

(3) subjecting said first sample of complex biological material to capillary electrophoresis without sieving matrix;

(4) following said capillary electrophoresis of said first sample, using a general detection method to detect the presence of compounds originating from said first sample of complex biological material and to produce a first detection pattern of compounds detected;

(5) providing a second sample of said complex biological material;

(6) combining said second sample of said complex biological material with said target to form a sample/target mixture;

(7) injecting an aliquot of said sample/target mixture into said apparatus for capillary electrophoresis without sieving matrix;

(8) subjecting said aliquot of said sample/target mixture to capillary electrophoresis without sieving matrix;

(9) following said capillary electrophoresis of said aliquot of said sample/target mixture, using said general detection method to detect the presence of compounds originating from said sample/target mixture and to produce a second detection pattern of compounds detected;

(10) comparing said first detection pattern to said second detection pattern to look for any compound represented in said first detection pattern that is not represented in said second detection pattern;

(11) isolating any compound originating from said complex biological material that is represented in said first detection pattern but is not represented in said second detection pattern; and

(12) determining whether said isolated compound has not been previously identified as interacting with said target.

5. The method according to claim 1 or claim 4, further comprising testing said isolated compound for therapeutic efficacy or pharmokinetic properties against said target.

6. A method of screening complex biological material for candidate, unidentified ligands of a selected target said method, comprising, in the order given, the steps of:

(1) providing a sample of complex biological material;

(2) combining said sample of complex biological material with said selected target to form a first, sample/target mixture;

(3) subsequently, combining said first mixture with a known, charged ligand of said target, to form a second, sample/target/known ligand mixture;

(4) injecting an aliquot of said second mixture into an apparatus for capillary electrophoresis without sieving matrix;

(5) subjecting said aliquot of said second mixture to capillary electrophoresis without sieving matrix;

(6) tracking the migration of said known ligand upon said capillary electrophoresis; and

(7) determining whether the migration pattern of said known ligand from step (6), when compared to a reference standard comprising the migration pattern of said known ligand in the presence of said target and the absence of said complex biological material, indicates the presence of a candidate unidentified ligand of said target, in said sample of complex biological material.

7. The method of claim 6 further comprising:

isolating said candidate unidentified ligand of said target, whose presence has been indicated in step (7), from said complex biological material;

determining whether said isolated compound has not been previously identified as interacting with said target; and

testing said isolated compound for therapeutic efficacy or pharmokinetic properties against said target.

8. The method of claim 6 wherein said known ligand is tight-binding.

9. A method of screening complex biological material for candidate, unidentified ligands of a selected target, said method comprising, in the order given, the steps of:

(1) providing a sample of complex biological material;

(2) combining said sample of complex biological material with said selected target to form a sample/target mixture;

(3) injecting an aliquot of said sample/target mixture into an apparatus for capillary electrophoresis without sieving matrix;